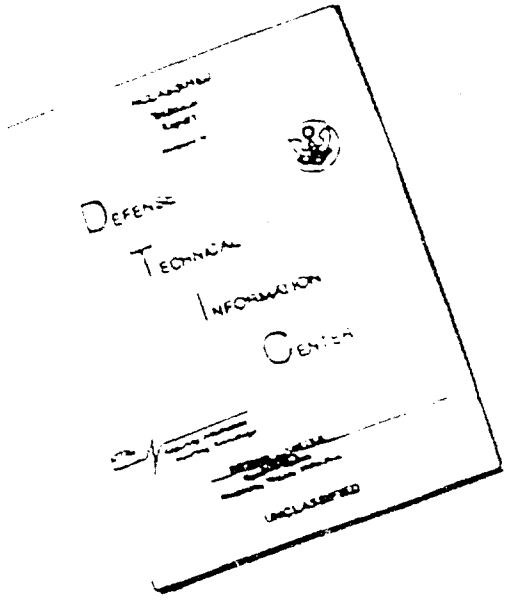
BIONOMICS

EG&G

DISCLAIMER NOTICE



THIS DOCUMENT IS BEST QUALITY AVAILABLE. THE COPY FURNISHED TO DTIC CONTAINED A SIGNIFICANT NUMBER OF PAGES WHICH DO NOT REPRODUCE LEGIBLY.

AD_____

12

ACUTE TOXICITY OF 1,3,5,7-tetranitro-octahydro-1,3,5,7-tetrazocine (HMX) TO AQUATIC ORGANISMS

BY

R.E. BENTLEY, G.A. LEBLANC, T.A. HOLLISTER, AND B.H. SLEIGHT, III

FINAL REPORT

APRIL, 1977

SUPPORTED BY

U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND

WASHINGTON, D.C. 20314

CONTRACT NO. DAMD-17-74-C-4101

E G & G

Project Officer
Mark C. Warner, Ph.D.

APPROVED FOR PUBLIC RELEASE: DISTRIBUTION UNLIMITED

UNCLASSIFIED

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

REPORT DOCUMENTATION PAGE

READ INSTRUCTIONS BEFORE COMPLETING FORM

1. REPORT NUMBER
2. GOVT ACCESSION NO.
3. RECIPIENT'S CATALOG NUMBER

4. TITLE (and Subtitle)
Acute Toxicity of HMX to Aquatic Organisms

5. TYPE OF REPORT & PERIOD COVERED
(7) Final Report,

6. PERFORMING ORG. REPORT NUMBER

7. AUTHOR(s)
(10) R.E. Bentley, G.A. LeBlanc, T.A. Hollister, B. H. Sleight, III

8. CONTRACT OR GRANT NUMBER(s)
(15) DAMD 17-74-C-4101

9. PERFORMING ORGANIZATION NAME AND ADDRESS
E G & G Bionomics
790 Main Street
Wareham, Massachusetts

10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS
(16) 62720A
3E762720A835 00.007
(17) 00

11. CONTROLLING OFFICE NAME AND ADDRESS
US Army Medical Research and Development Command
Washington, D. C. 20314

12. REPORT DATE
(11) April 1977

13. NUMBER OF PAGES
23

14. MONITORING AGENCY NAME & ADDRESS (if different from Controlling Office)
(12) 29 p.

15. SECURITY CLASS. (of this report)
Unclassified

15a. DECLASSIFICATION/DOWNGRADING SCHEDULE

16. DISTRIBUTION STATEMENT (of this Report)
Approved for public release; distribution unlimited

17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)
(6) Acute Toxicity of 1,3,5,7-tetranitrooctahydro-1,3,5,7-tetrazocine (HMX) to Aquatic Organisms.

18. SUPPLEMENTARY NOTES

19. KEY WORDS (Continue on reverse side if necessary and identify by block number)
Toxicity, 1,3,5,7-tetranitrooctahydro-1,3,5,7-tetrazocine, HMC, Aquatic, Algae, Water Flea, Scud, Sowbug, Midge, Channel Catfish, Rainbow Trout, Fathead minnow, Water Quality Criteria, Evaluation.

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)
The acute toxicity of 1,3,5,7-tetranitrooctahydro-1,3,5,7-tetrazocine (HMX) was studied utilizing aquatic organisms representing several different trophic levels in aquatic ecosystems. Generally, no adverse effects of exposure to 32 mg/ℓ HMX were observed among any of the algae, fish, or invertebrate species tested. The 7-day old fry of the fathead minnow were the only life stage or species acutely affected. Based on an application factor of 0.05 and a 96-hour LC50 for the most sensitive aquatic organism (7-day old fry

DD FORM 1 JAN 73 1473 EDITION OF 1 NOV 65 IS OBSOLETE

UNCLASSIFIED

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

of the fathead minnow) tested (15 mg/ℓ), a water quality criterion of 0.75 mg/ℓ is proposed for the protection of freshwater aquatic life with an adequate margin of safety.

SUMMARY

The concentration of HMX acutely toxic to four species of algae, four species of freshwater fishes and four species of invertebrates were clearly shown to be greater than 32 mg/ℓ (ppm). Generally, no adverse effects of exposure to 32 mg/ℓ HMX were observed among any of the algae, fish or invertebrate species tested. The 7-day old fry of the fathead minnow were the only life stage or species acutely effected (96-hour LC50, 15 mg/ℓ).

Based on these acute toxicity data, and the lack of any evidence suggesting that HMX is cumulatively toxic to aquatic organisms, the use of a general application factor of 0.05 is proposed for estimating the safe concentrations of HMX in aquatic ecosystems. Using a general application factor (0.05) and the 96 hour LC50 for the most sensitive aquatic organism (7-day old fry of the fathead minnow) tested (15 mg/ℓ), a water quality criterion of 0.75 mg/ℓ is proposed for the protection of freshwater aquatic life with an adequate margin of safety.

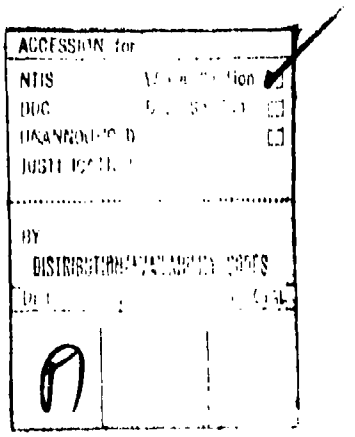


TABLE OF CONTENTS

INTRODUCTION

1,3,5,7-tetranitrooctahydro-1,3,5,7-tetrazocine (HMX) is known to occur in discharges from the Holston Army Ammunition Plant in Kingsport, Tennessee. Rosenblatt et al. (1973) reported a solubility of HMX in water at 25°C of 2 mg/ℓ, while Warner (1977, pers. comm.) reported a solubility of HMX in water of 6.6 mg/ℓ at 20°C. Water and Air Research (1976) projected the discharge of HMX from proposed facility X at 0.3-0.8 mg/ℓ under normal operating conditions and under the worst operating conditions would not exceed 1.4 mg/ℓ. Since there appears to be little information on the toxicity of HMX on aquatic organisms, studies were initiated to investigate the acute toxicity of HMX on aquatic organisms. The objective of the program was to provide the data base required to perform a hazard evaluation relative to the occurrence of HMX in the aquatic environment, and to recommend a proposed water quality criteria for HMX for the protection of freshwater aquatic life with an ample margin of safety.

The specific efforts undertaken included investigations of: (a) the acute toxicity of HMX to a wide variety of aquatic organisms representing all trophic levels under static conditions and (b) the effects of variations in water quality on the acute toxicity of HMX to fish.

The studies to evaluate the acute toxicity of HMX to phytoplankton were performed at the Marine Research Laboratory of E G & G, Bionomics in Pensacola, Florida. The studies to evaluate the toxicity of HMX to all other aquatic organisms were conducted at the Aquatic Toxicology Laboratory of E G & G, Bionomics in Wareham, Massachusetts.

MATERIALS AND METHODS

Test Material

The 1,3,5,7-tetranitrooctahydro-1,3,5,7-tetrazocine (HMX) used in these studies was obtained from the Holston Army Ammunition Plant in Kingsport, Tennessee. For all phytoplankton tests, the HMX was added directly to the test medium. For all static, acute toxicity tests, the HMX was dried and mixed with acetone to form a superstock solution. Concentrations of HMX are reported as milligrams (mg) per liter (ℓ) of diluent water, or parts per million (ppm).

Test Organisms

Algae tested were the cyanophytes (blue-green) Microcystis aeruginosa and Anabeana flos-aquae; the chlorophyte (green) Selenastrum capricornutum; and the chrysophyte (diatom) Navicula pelliculosa. Cultures were obtained from the collection at the University of Indiana, Bloomington, Indiana, and the Pacific Northwest Water Quality Laboratory (EPA, Corvallis, Oregon). Each species was maintained in stock cultures at Bionomics Marine Research Laboratory. Culture medium was prepared according to the formula described in "Algal Assay Procedure: Bottle Test" (U.S. EPA, 1971).

Macroinvertebrates exposed to HMX were water flea (Daphnia magna, scud (Gammarus fasciatus), sowbug (Asellus militaris), and midge (Chironomus tentans). The water flea were acquired from Bionomics' laboratory cultures, and the scud, sowbug, and midge were collected in the Wareham, Massachusetts area by Bionomics' personnel.

At the initiation of testing, water flea were 0-24 hours old, scud and sowbug were in the juvenile stage; and the midge larvae were in the second or third instar range.

Fish utilized in the acute, static toxicity tests were bluegill (Lepomis macrochirus), rainbow trout (Salmo gairdneri), channel catfish (Ictalurus punctatus), and fathead minnow (Pimephales promelas). Unless otherwise noted, the bluegill were acquired from a commercial fish farmer in Nebraska and had a mean ($\pm$ S.D.) weight of 1.0 ($\pm$ 0.3) g and a mean ($\pm$ S.D.) standard length of 35 ($\pm$ 6) mm. The rainbow trout were acquired from a commercial trout producer in Massachusetts and had a mean weight and length of 0.9 ($\pm$ 0.3) g and 43 ($\pm$ 4) mm, respectively. The channel catfish were obtained from a fish farmer in Arkansas and had a mean weight of 1.2 ($\pm$ 0.5) g and a mean length of 57 ($\pm$ 11) mm. The fathead minnow were obtained from a commercial producer in Arkansas, and had a mean weight of 1.0 ($\pm$ 0.4) g and a mean length of 43 ($\pm$ 8) mm. Thirty fish representative of test populations of each species were weighed and measured for the calculation of means and standard deviations for each group.

Prior to use in tests, all fish were held in 1700-ℓ concrete raceways which were coated with an epoxy resin paint to prevent leaching of materials into the water. Flow of well water (temperature, 20 ± 1.0°C for bluegill, channel catfish, and fathead minnow, and 14 ± 1.0°C for the rainbow trout; hardness 35 mg/ℓ as $CaCO_3$; pH 7.1, and dissolved oxygen concentration, >60% of saturation) into these raceways was at a minimum of 4 ℓ/minute, which provided an adequate water turnover rate for holding these species. The fishes were maintained in these laboratory hatchery facilities for at least thirty days prior to use in bioassays. During this period, cumulative mortality for each species was <2%; no mortality was observed during the 48 hours immediately prior to testing, and these fishes were judged to be in excellent condition. Fish of each species were from the same year class, and the standard length of the longest fish was no more than twice that of the shortest.

Test Methods

In order to evaluate the relative susceptibility of a broad spectrum of aquatic organisms to HMX, static bioassays were conducted. Due to the lack of solubility in water of HMX at any concentration greater than 6.6 mg/ℓ at 20°C (Warner, 1977 pers. comm.) it was decided to establish a high concentration of 32 mg/ℓ.

During all bioassays to investigate the acute toxicity of HMX to aquatic organisms, two series of concentrations were established within each bioassay, a series of range-finding concentrations (preliminary test) and a series of definitive concentrations (definitive test). The preliminary test was conducted to determine an approximate range of concentrations for evaluating the dose-response relationship. The definitive test, consisting of at least five concentrations, evaluated the dose-response relationship to a degree allowing the median effective concentration (EC50) or the median lethal concentration (LC50) to be calculated from the data with optimum accuracy.

Algal assays were conducted according to the method described in "Algal Assay Procedure: Bottle Test" (U.S. EPA, 1971). To determine the effects of HMX on algae, measurements were made of the chlorophyll a content of exposed and control cultures of each of the four test species. In addition, to confirm these results, determinations of cell numbers for cultures of M. aeruginosa, S. capricornutum and N. pelliculosa and of optical density of A. flos-aquae were performed.

Chlorophyll a analyses were conducted according to the procedures of Strickland and Parsons (1968) and involved filtering algal cultures from test medium, extracting chlorophyll by treatment of algal cells with acetone, determining extinction values

with a spectrophotometer and finally, calculating the chlorophyll a concentration in the solution. Chlorophyll a and optical density measurements (at 680 nanometers) were made with a Bausch & Lomb Spectronic 20 spectrophotometer. Cell counts were performed with a compound light microscope and a hemacytometer. In each case, the measurements obtained from duplicate exposed cultures were averaged, the results compared with those from duplicated controls and a percentage effect (relative to controls) was calculated.

Each test concentration was converted to its logarithms and the corresponding percentage effect (change in chlorophyll a concentration, optical density or cell number) converted to a probit. The 24-, 48-, and 96-hour median effective concentrations, EC50's (concentrations effective in changing the chlorophyll a concentration, optical density or cell number of exposed algae by 50% as compared to controls) and their 95% confidence limits were then estimated from a linear regression equation calculated with a programmable calculator.

Test methods used for static bioassays with macroinvertebrates and fishes were as described in "Methods for Acute Toxicity Tests with Fish, Macroinvertebrates, and Amphibians" (U.S. EPA, 1975).

Results of macroinvertebrate and fish toxicity tests are expressed as LC50's (concentrations lethal to 50% of test animals). The LC50 values and their 95% confidence limits

were estimated from a linear regression equation calculated with a programmable calculator. Data from replicates were averaged and utilized in the regression analysis.

Macroinvertebrate bioassays were conducted in 250-ml beakers containing 200 ml of solution at 20 ± 1.0°C. Aged well water (hardness, 35 mg/ℓ as $CaCO_3$; pH, 7.1) was utilized in the performance of these bioassays.

Dissolved oxygen values in test vessels during static bioassays with invertebrates ranged from 8.0 to 8.2 mg/ℓ throughout the testing period. Macroinvertebrates were introduced into test beakers within 30 minutes following addition of the HMX; 15 animals in each species were tested at each concentration (3 replicates, 5 animals/replicate). Static fish bioassays were conducted in 19.6-liter glass vessels containing 15 liters of water, and were held in constant temperature water baths at 20 ± 1.0°C for the bluegill, channel catfish, and fathead minnow, and at 10 ± 1.0°C for the rainbow trout. The standard diluent (well water) for the fish species had a hardness of 35 mg/ℓ as $CaCO_3$ and a pH of 7.1. Dissolved oxygen values in various test vessels during bioassays with fishes ranged from 9.0 initially to 4.0 mg/ℓ at the end of the tests. Fish were introduced into each test vessel within 30 minutes after the compound was added; 30 animals of each species were utilized for each concentration (3 replicates, 10 animals/replicate).

Fathead minnows were chosen as the test species to evaluate the relative susceptibility of life stages of fish to HMX because of the ability to readily procure their various life stages in the laboratory. The susceptibility of selected life stages (egg, 1-hour old newly-hatched fry, 7-day old fry, 30- day old fry, and 60-day old fry) of fathead minnow (Pimephales promelas) to HMX was evaluated under static bioassay conditions for a 144-hour period with the eggs, and for a 96-hour period with all other life stages. The egg, 1-hour old fry and 7-day old fry bioassays were conducted in 250-ml beakers containing 200 ml of solution (10 animals/beaker, 3 replicates/concentration, 30 animals/concentration). The 30-day old fry and 60-day old fry bioassays were conducted in 1-gallon glass jars containing 3 ℓ of solution (10 fry-jar, 3 replicates/concentration, 30 animals/concentration). The LC50 values for the egg tests were calculated at 24, 48, 96 and 144 hours. The time period of 144 hours allowed 100% hatch of eggs in all control beakers. In addition to percent mortalities, percent hatch of eggs was also observed. These tests were conducted at 25 $\pm$ 1.0°C, and the standard diluent had a pH of 7.1 and total hardness (EDTA) of 35 mg/ℓ as $CaCO_3$.

Due to their sensitivity to the chemicals, their availability, and their expected presence in most of those areas where HMX might be found, bluegill were selected as the test species

to evaluate the effect of water quality on the toxicity of HMX. The susceptibility of bluegill to HMX under various water quality conditions was evaluated during static bioassays for a 96-hour period. The bluegill used in these tests were obtained from a commercial fish farmer in Nebraska, and had a mean ($\pm$S.D.) wet weight and standard length of 0.9 ($\pm$ 0.2) g and 33 ($\pm$ 5) mm, respectively. Bioassays were conducted utilizing bluegill to determine the 24, 48 and 96 hour LC50 values of HMX: (a) at three temperatures representing the lower end (15°C), mid-point (20°C), and upper end (25°C) of the normal temperature range for bluegill using soft water (35 mg/ℓ $CaCO_3$) at neutral pH; (b) in soft water (35 mg/ℓ $CaCO_3$), in hard water (100 mg/ℓ) and in very hard water (250 mg/ℓ $CaCO_3$) using water of pH 7.0 at the recommended test temperature of 20°C; and (c) at pH's of 6.0, 7.0 and 8.0 urging standard soft water at the recommended test temperature of 20°C. The diluent for each of these conditions was prepared according to the procedures recommended by Marking and Dawson (1973). Dissolved oxygen values in various test vessels during these bioassays ranged from 9.0 initially to 4.2 mg/ℓ at the end of the tests.

General availability and their expected presence in those areas where the HMX might be found resulted in the use of bluegill to evaluate the stability of the toxicological properties of HMX. The susceptibility (LC50) of bluegill (Lepomis macrochirus) to HMX was evaluated under static bioassay conditions for a 96-hour period utilizing solutions

which were "aged" for 0, 12, 24, 48 and 96 hours. Fish (10 fish/replicate, 3 replicates/concentration, 30 fish/concentration) were introduced into aged test solution at each time period. The bluegill used in these tests were acquired from a commercial fish farmer in Nebraska, and had a mean ($\pm$ S.D.) wet weight of 0.8 ($\pm$ 0.2) g and a mean ($\pm$ S.D.) standard length of 32 ($\pm$ 4) mm. The standard diluent had a pH of 7.1 and a total hardness (EDTA) of 35 mg/ℓ as $CaCO_3$.

RESULTS

The effects of the exposure to HMX on the number of cells or optical density of the phytoplankters tested (Table 1), and on the chlorophyll *a* concentrations of phytoplankters (Table 2) are summarized. As is evident from these data, the EC50 values for HMX and all four phytoplankton species are >32 mg/l irrespective of the criteria selected for measuring effects of exposure. The data indicate an increase of cells/ml or chlorophyll *a* utilizing all species of algae. *Navicula pelliculosa* exhibited the greatest increase (18%) in cells/ml, while *Anabeana flos-aquae* exhibited an increase of 39% in chlorophyll *a* content at 10 mg/l HMX.

Neither the invertebrate species nor the fish species were affected during 96 hours exposure to nominal HMX concentrations as high as 32 mg/l (Table 3); therefore, LC50 values for HMX and these species are >32 mg/l.

The acute toxicity of HMX to various life stages of the fathead minnow indicated virtually no adverse effect of exposure with on exception (Table 4). The 7-day old fry exhibited the greatest sensitivity to acute exposure (LC50 at 96 hour, 15 mg/l). A dose-response was evident at all concentrations tested indicating that the HMX was more soluble than the reported 6.6 mg/l.

As a result of this data, all concentrations are reported as nominal rather than measured.

The results of the bioassays to determine the effects of varying water quality on the toxicity of HMX indicate that none of the parameters tested had an effect on the acute toxicity (Table 5). Similarly, aging solutions of HMX up to 96 hours had no effect on the toxicity to bluegill (Table 6).

DISCUSSION AND CRITERIA FORMULATION

The results of these studies indicate that HMX presents relatively little hazard to freshwater aquatic life. A nominal concentration (32 mg/l) ca 23X higher than the maximum concentration which might be expected in freshwaters receiving the effluents from munitions plants (1.4 mg/ℓ) was not acutely toxic (i.e., did not affect 50% or more of exposed test organisms) to all four species of algae, all four species of invertebrates, and all four species of fish. Of the critical life stages tested, only the 7-day old fry exhibited a susceptibility to HMX. Furthermore, results of tests conducted with bluegill exposed to HMX under various water quality conditions (i.e., three temperatures, three hardnesses and three pH's) indicated that HMX at nominal concentrations to 32 mg/ℓ was non-toxic to these fish even under altered water quality conditions. Aging solutions up to 96 hours had no effect on the toxicity of HMX to fish at nominal concentrations to 32 mg/ℓ.

Based on the 96-hour LC50 estimated for the most sensitive species tested, we feel it is appropriate to use an application factor of 0.05 to estimate the concentration in receiving water which would not be expected to produce any significant deleterious effects on aquatic life. Multiplying the estimated 96-hour LC50 of 15 mg/ℓ (7-day old fathead minnow fry) by the 0.05 ap-

plication factor, a water quality criterion of 0.75 mg/ℓ HMX should provide reasonable protection of aquatic life.

LITERATURE CITED

Marking, L.L. and V.K. Dawson. 1973. Toxicity of quinaldine sulfate to fish. Invest. Fish Control No. 48. U.S. Fish Wildl. Serv., Washington, D.C. 8 p.

Rosenblatt, D.H., M.T. Small and J.J. Barkely. 1973. Munitions production products of potential concern as waterborne pollutants - Phase I. U.S. Army Medical Environmental Engineering Research Unit, Edgewood Arsenal, Maryland. AD 912752. 81 pp.

Strickland, J.D.H., and T.R. Parsons. 1968. A Practical Handbook of Seawater Analysis. Fish. Res. Bd. Canada. Ottawa Bull. 167 (Second Edition). 310 p.

Water and Air Research, Inc. 1976. Water Quality Assessment for the Proposed RDX-HMX Facility - McAllister Naval Ammunition Depot. Vol. 1. AD #A026394. 139 pp.

U.S. Environmental Protection Agency. 1971. Algal Assay Procedure: Bottle Test. National Eutrophication Research Program, Pacific Northwest Water Quality Laboratory, Corvalis, Oregon. 82 pp.

U.S. Environmental Protection Agency. 1975. Methods for Acute Toxicity Tests with Fish, Macroinvertebrates and Amphibians. Ecological Research Series, EPA-660/3-75-009. 61 pp.

Table 1 -- Percent change[a] in the cell density[b] of Selenastrum capricornutum, Microcystis aeruginosa, Anabeana flos-aquae and Navicula pelliculosa after 96 hours exposure to HMX.

Nominal HMX concentration (mg/ℓ)	S. capricornutum	M. aeruginosa	A. flos-aquae	N. pelliculosa
0.32	0	0	0	0
1.0	0	0	0	+3
3.2	0	+3	0	+8
10	+3	+4	+7	+12
32	+7	0	+10	+18

a Percent change is relative to control cultures.

b Determined by cell counts for all species except A. flos-aquae which was determined by optical density.

Table 2 -- Percent change[a] in the chlorophyll a content of Selenastrum capricornutum, Microcystis aeruginosa, Anabeana flos-aquae and Navicula pelliculosa after 96 hours exposure to HMX.

Nominal HMX concentration (mg/ℓ)	S. capricornutum	M. aeruginosa	A. flos-aquae	N. pelliculosa
0.32	0	0	+7	0
1.0	0	0	+5	+4
3.2	+3	+3	+12	+11
10	+9	+8	+39	+9
32	+8	+8	+24	+23

[a] Percent change is relative to control cultures.

Table 3 -- Acute toxicity values[a] (mg/ℓ) for HMX utilizing aquatic invertebrates and fishes determined during static bioassays.

Species	Hours of exposure		
	24	48	96
Daphnia magna (water flea)	>32	>32	-
Gammarus fasciatus (scud)	>32	>32	-
Asellus militaris (sowbug)	>32	>32	-
Chironomus tentans (midge)	>32	>32	-
Lepomis macrochirus (bluegill)	>32	>32	>32
Salmo gairdneri (rainbow trout)	>32	>32	>32
Ictalurus punctatus (channel catfish)	>32	>32	>32
Pimephales promelas (fathead minnow)	>32	>32	>32

[a] Acute toxicity values are expressed as effective concentrations causing immobilization (EC50) after 24 and 48 hours for invertebrates and lethal concentrations (LC50) after 24, 48 and 96 hours for fishes.

Table 4 -- Acute toxicity of HMX to selected life stages of fathead minnows (Pimephales promelas) as determined during static bioassays.

Life stage	LC50 (mg/ℓ) 24-hour	48-hour	96-hour	144-hour
eggs	>32	>32	>32	>32
1-hour post hatch	>32	>32	>32	-[a]
7-days post hatch	>32	25 (7.6-81)[b]	15 (8.8-26)	-
30-days post hatch	>32	>32	>32	-
60-days post hatch	>32	>32	>32	-

[a] Egg testing conducted for 144 hours; all other life stages conducted for 96 hours.

[b] 95% confidence interval.

Table 5 -- Acute toxicity of HMX to bluegill (Lepomis macrochirus) under varying conditions of water quality during static bioassays.

Temperature (°C)	pH	Harndess (mg/ℓ $CaCO_3$)	96-hour LC50 (mg/ℓ)
15	7.0	35	>32
20	7.0	35	>32
25	7.0	35	>32
20	7.0	35	>32
20	7.0	100	>32
20	7.0	250	>32
20	6.0	35	>32
20	7.0	35	>32
20	8.0	35	>32

Table 6 -- Acute toxicity of aged solutions of HMX to bluegill (Lepomis macrochirus) during static bioassays.

Age of solutions prior to bioassay (hrs.)	96-hour LC50 (mg/ℓ)
0	>32
12	>32
24	>32
48	>32
96	>32